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Note

Characterisation of lactic acid bacteria isolated from artisanal Egyptian Ras cheese

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Abstract – Wild LAB (188 strains) isolated from artisanal Egyptian Ras cheese including *Lactococcus* (15), *Lactobacillus* (95), *Enterococcus* (77) and *Pediococcus* (1) were characterised for biochemical and production characteristics related to their technological performance. All *Lactococcus* (subsp. *lactis* and *cremoris*) were able to grow at 40 °C and in the presence of 4% NaCl. Six *L. lactis* subsp. *lactis*, one strain of subsp. *cremoris* and 95% of *Lactobacillus* strains were salt-tolerant, as they were able to grow in 6.5% NaCl. Twenty percent of lactococci, 21% of lactobacilli and 43% of enterococci showed a medium acidification rate, one strain of *Lb. delbrueckii* subsp. *lactis* and *E. faecium* exhibited a fast acidifying ability. Most lactobacilli revealed higher aminopeptidase and autolytic activity when compared with other strains. Twenty-seven percent of tested strains appeared to have antimicrobial activity and the highest inhibitory activity was observed among enterococci (13% of 188 strains). Exopolysaccharides (capsule) were detected in 4.3% of the lactobacilli. Most strains were able to produce a high yield of biomass after growth, showed a good biomass separation and were not affected by lyophilisation treatment. Strains which showed outstanding characteristics were screened for their flavour-forming abilities in milk cultures. Ninety-one strains, 7 *Lactococcus*, 52 *Lactobacillus* and 32 *Enterococcus*, were selected for an application in Ras cheese according to their production and technological criteria: salt tolerance, ability to grow at 40–45 °C, acid production, proteolytic activity, flavour and bacteriocin production. These strains are currently being tested in pilot-scale cheese trials to improve Egyptian Ras cheese.

Egyptian Ras cheese / starter culture / wild LAB / acidification / aminopeptidase / autolysis / bacteriocin / exopolysaccharide / flavour

摘要 - 从手工制作埃及拉斯干酪中分离出的乳酸菌特性研究。从手工制作的埃及拉斯干酪 (Ras cheese) 中共分离出 188 株野生乳酸菌，根据这些菌株的生化和生长特性确定这些菌株分别为乳球菌 (*Lactococcus*) 15 株，乳杆菌 (*Lactobacillus*) 95 株，肠球菌 (*Enterococcus*) 77 株，片球菌 (*Pediococcus*) 1 株。所有的乳球菌 (乳酸亚种和乳脂亚种) 都可以在 40 °C 和 4% NaCl 的环境中生长。其中 6 株乳酸乳球菌乳亚种 (*Lc. lactis* subsp. *lactis*)、1 株乳酸乳球菌乳脂亚种 (*Lc. lactis.* subsp. *cremoris*) 和 95% 的乳杆菌具有耐盐性，可以在 6.5% NaCl 环境中生长。 20% 的乳球菌、 21% 的乳杆菌和 43% 的肠球菌产酸速率适中，其中一株德式乳杆菌乳亚种 (*Lb. delbrueckii* subsp. *lactis)* 和一株屎肠球菌 (*E. faecium*) 的产酸速率较快。与其他乳酸菌相比大多数乳杆菌分泌氨基肽酶活力较高，菌体的自溶能力较强。实验证明 27% 受试菌株具有明显的抗菌活性，其中肠球菌 (占 188 株的 13%) 的抑菌活力最高。 4.3% 的乳

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杆菌能够分泌胞外多糖（荚膜多糖）。经体外培养后大多数菌株生长状态良好，生物量较高，分离后的菌体经过冻干处理后，活性没有受到影响。将那些具有突出特性的菌株在乳基培养基中单独培养，从中筛选出具有显著产香能力的菌株。根据标准的拉斯干酪生产工艺和技术要求，将这些菌株应用于拉斯干酪的生产中，以耐盐性、在 40 ～ 45 °C 下的生长情况、产酸量、蛋白水解能力、产香能力和产细菌素能力作为评价指标，从中筛选出 91 株乳酸菌，其中乳球菌 7 株、乳杆菌 52 株、肠球菌 32 株。这些菌株已经用于中试规模的研究中，用以改进埃及拉斯干酪的品质特性。

埃及拉斯干酪 / 发酵剂 / 野生乳杆菌 / 酸化 / 氨基肽酶 / 自溶 / 细菌素 / 胞外多糖 / 风味

Résumé – Caractérisation de bactéries lactiques isolées à partir du fromage traditionnel égyptien « Ras ». Des souches sauvages de *Lactococcus*, *Lactobacillus*, *Enterococcus* et *Pediococcus* ont été isolées à partir du fromage égyptien « Ras ». Les souches furent ensuite caractérisées d'après leur aptitude technologique et biochimique. *Lactococcus lactis* subsp. *lactis* et *cremoris* se développaient à 40 °C en présence de 4 % Nacl. Six souches *de Lactococcus lactis* subsp. *lactis* et une souche de *Lactococcus lactis* subsp. *cremoris* ainsi que 95 % des souches de *Lactobacillus* étaient résistantes au chlorure de sodium à des concentrations de 6,5 %. Environ 20 % des lactocoques, 21 % des lactobacilles et 43 % des entérocoques montraient une activité acidifiante moyenne. La majorité des lactobacilles possédaient une forte activité autolytique et aminopeptidasique. Vingt-sept pourcent des souches testées montraient une activité inhibitrice vis-à-vis des autres souches. La plus forte activité inhibitrice a été révélée chez les entérocoques. La production d'exopolysaccharides a été démontrée chez 4,8 % des lactobacilles. En règle générale, les souches qui montraient des caractéristiques intéressantes ont ensuite était sélectionnées pour la production de composés d'arôme dans le lait. Quatre-vingt-onze souches ont été isolées et sélectionnées pour la fabrication de fromage Ras en fonction de leur tolérance au sel, croissance à 40–45 °C, production d'acide, activité protéolytique, production d'arômes et de bactériocines. Ces souches seront testées à l'échelle pilote pour fabriquer du fromage Ras.

fromage Ras / souche sauvage / bactérie lactique / levain / acidification / aminopeptidase / autolyse / bactériocine / exopolysaccharide / flaveur

1. INTRODUCTION

Ras cheese is the main traditional hard cheese in Egypt; it is similar to the Greek variety "Kefalotyri" [33]. A full description of the manufacture, ripening and chemical composition of Ras cheese is reviewed by Abou-Donia [1]. Generally, it is manufactured in a high proportion under artisanal conditions, mainly from raw cow's milk or a mixture of cow's and buffalo's milk without using starter cultures [26]. A mixture of cow's and goat's milk has also been used for making Ras cheese [15]. In such production, fermentation and ripening occurs by the interaction of different wild flora present in raw milk and the surrounding environment. Therefore, the "biodiversity" of LAB involved in cheese production is considered a fundamental factor for the maintenance of the characteristic features and quality of Ras cheese.

Recently, the new Egyptian standards for hard cheese, 2001 [14], published by the Egyptian organisation for standardisation and quality control, indicated that Ras cheese must be made from pasteurised milk. These standards are aiming to produce cheese under improved hygienic conditions, showing consistent and better quality. That means starter cultures should be added to pasteurised milk prior to the cheese manufacture. Therefore, there is a great need for new starter cultures to use in the production of Ras cheese.

Identification, characterisation and selection of wild LAB from traditional cheese made all around the world in a natural way has been considered in the search for new industrially important cultures [4, 7, 41]. Often, wild strains are adapted to specialised niches, which have evolved with minor or major changes of the genotype and phenotype, in dairy industries. The use of such strains offers great potential for new applications or for existing processes [21].

In our university, research work was aimed at improving the manufacture of Ras cheese. The typical Ras cheeses made from raw milk were evaluated according to their sensory evaluation, rheological, physicochemical and microbiological characteristics and flavour formation to be able to manufacture a typical Ras cheese from pasteurised milk and starter cultures [3, 6]. For the development of new starter cultures suitable for Ras cheese, several wild LAB were isolated from market Ras cheese and identified, then kept in the culture collection of the Faculty of Agriculture, Alexandria University (FAAU) [17]. The aim of the present work is to characterise LAB associated with artisanal Egyptian Ras cheese with unique features such as salt tolerance, proteolytic activity, acid production, aroma and bacteriocin production in order to select them as starter cultures to improve Ras cheese flavour.

2. MATERIALS AND METHODS

2.1. Origin of strains

A total of 188 wild LAB strains obtained from the culture collection of FAAU were tested in this study. All strains originated from traditional Ras cheese with artisanal production from local factories in the delta region. These strains belong to several genera of LAB (Tab. I); *Lactococcus* and *Enterococcus* strains were grown in M17 medium (Biolife, Milano, Italy; Terzaghi and Sandine [39]), while *Lactobacillus* and *Pediococcus* were grown in MRS medium (Biolife, Milano, Italy; De Man et al. [13]), at the optimum growth temperature; 30 °C for *Lactococcus*, 30–37 °C for mesophilic lactobacilli and 42 °C for thermophilic lactobacilli.

2.2. Technological characteristics of strains

2.2.1. Growth characteristics of strains

The ability of strains to grow at 10, 30, 40 and 45 °C was examined in suitable medium (see above) for 48 h. The growth was followed by measuring the optical density at 650 nm (OD_{650}) using a spectrophotometer (NOVA SPEC II, pharmacia LKB Biotech., UK). The sensitivity to salt of the strains was determined by following the growth of strains in suitable medium at optimum growth temperatures in the presence of 1, 2, 4 and 6.5% NaCl. The production of carbon dioxide from glucose was carried out; 1 mL of the strain was inoculated in 2 mL MRS agar at 40–45 °C; after 1 h, 2 mL agar (15%) was added as an agar plug and incubated at 37 °C, then observed for gas production after 2–4 d. A control was carried out using a yeast, *Saccharomyces lactis* N.C.Y.C. 571, known for the production of carbon dioxide.

2.2.2. Acidification activity

The acidification rate was measured by the change in pH during time in reconstituted skim milk (RSM) at an appropriate temperature. The cultures were considered as fast-, medium- or slow-acidifying when a ΔpH of 0.4 units was achieved after 3, 3–5 and > 5 h, respectively.

2.2.3. Aminopeptidase (AP) activity

The strains were cultivated and centrifuged (10 min, 4000× *g*, 4 °C), the pellets washed twice and resuspended in potassium phosphate buffer (10 mmol·L^{-1}, pH 7.0) and diluted to OD_{650} = 1.0 (*ca.* 100 mg cell wet weight·mL^{-1}). Cell permeabilisation was carried out as described by Miozzari et al. [31] and the AP activity was measured according to El-Soda and Desmazeaud [18].

2.2.4. Autolytic activity

The rate of cell autolysis was measured as described by Thiboutot et al. [40].

2.2.5. Antagonistic activity

Antagonistic activity was examined as described by Geis et al. [23]. Cultures belonging to the same genus were interacted together. Each tested strain was applied as inhibitor while another one was taken as an indicator organism.

Table I. Growth characteristics of strains.

	Total	Growth at			Growth with NaCl		CO_2 production
		10	40	45 °C	4%	6.5%	
Lactococcus	**15**						
L. lactis subsp. *lactis*	13						
	(6)	+	+	–	+	+	–
	(7)	+	+	–	+	–	–
L. lactis subsp. *cremoris*	2						
	(1)	+	+	–	+	–	–
	(1)	+	+	–	+	+	–
Lactobacillus	**95**						
Lb. delbrueckii subsp. *lactis*	5	–	+	+	+	–	–
Lb. fermentum	16						
	(11)	–	+	+	+	+	+
	(4)	–	+	+	–	–	+
	(1)	+	+	+	+	–	+
Lb. rhamnosus	31	+	+	+	+	+	–
Lb. paracasei subsp. *paracasei*	6						
	(5)	+	+	+	+	+	–
	(1)	+	+	–	+	+	–
Lb. pentosus	10	+	+	–	+	+	–
Lb. plantarum	21						
	(12)	+	+	–	+	+	–
	(5)	+	+	+	+	+	–
	(1)	+	+	+	+	+	+
	(1)	+	+	–	+	+	+
	(2)	–	+	–	+	+	+
Lb. brevis	3	+	+	–	+	+	+
Lb. acidophilus	1	+	+	+	+	+	–
Lb. salivarius	2						
	(1)	–	+	+	+	+	+
	(1)	–	+	–	+	+	–
Enterococcus	**77**						
E. faecium	59	+	+	+	+	+	–
E. durans	5	+	+	+	+	–	–
E. faecalis	9	+	+	+	+	+	–
E. avium	2	+	+	+	+	+	–
Pediococcus	**1**						
Ped. pentosaceus	1	+	+	+	+	–	–
Total	**188**						

2.2.6. Exopolysaccharide (EPS) production

Strains were tested for slime formation using the inoculated loop method [29]. The positive strains were tested for capsule production with phase contrast microscopy (Zeiss Microscope, Germany) according to the method of Prescoll et al. [34].

2.3. Production characteristics of strains

Biomass production was determined as described by Gerhardt [24]. According to the cell dry weight (CDW $mg \cdot L^{-1}$), the strains were divided into three groups; good ≥ 1.30 $mg \cdot L^{-1}$, fair 1.29–0.6 $mg \cdot L^{-1}$ and poor < 0.6 $mg \cdot L^{-1}$.

The optical density of the supernatant that resulted after the centrifugation (10 min, 4000× *g*, 4 °C) of the growth media was measured at 650 nm and used to express the biomass separation; a zero reading was taken as an indication for excellent separation, OD_{650}(0–0.1) indicated a good separation, OD_{650}(0.2–0.3) and more than 0.3 indicated a fair and poor separation, respectively.

The obtained pellets were resuspended in RSM (10% w/v) fortified with 7% sucrose and were subjected to freeze-drying using a Labconco Freeze-dryer system (Labconco, MO, USA) as described by El-Soda et al. [20] to determine the stability of lyophilised cultures. The acidification activity of freeze-dried and untreated cultures was followed and the cultures were grouped into three classes (good, ≤ 1 h; fair, ≤ 1.5 h; poor, >2 h) depending on the difference in the required time needed to drop the pH by 0.4 units.

The strains were tested in triplicate for the various above characteristics.

2.4. Flavour production

Individual strains were pre-grown for 16 h at 30 °C in RSM (10% w/v) containing 0.1% yeast extract; 1% of each culture was added to 100 mL skimmed UHT milk. Sensory evaluation was carried out after incubation at an optimum temperature for 48 h by 7 to 10 graders. The intensity of flavour attributes was scored on a scale of: 1, slightly; 2, moderate; 3, strong; 4, very strong.

3. RESULTS AND DISCUSSION

3.1. Technological characteristics of strains

LAB isolated from artisanal Ras cheese (188 strains), listed in Table I, were characterised; *Lactobacillus* (50.5%) and *Enterococcus* (41%) were the predominant genera, with *Lactococcus* (8%) and *Pediococcus* (0.5%) being associated with Ras cheese. Several species of lactobacilli were present; *Lb. rhamnosus* 32.6%, *Lb. plantarum* 22.1%, *Lb. fermentum* 16.8%, *Lb. pentosus* 10.5% and other species 18.0%. A high number of enterococci were present in Ras cheese; *E. faecium* (76.6%) was the predominant species, *E. faecalis* (11.7%) and other species represented 11.7% of the strains. However, in several cheeses produced from raw milk, enterococci were found to have a relevant role in the ripening process [22, 36]. From a technological point of view, certain species seem to play a fundamental role in the production of typical artisanal Ras cheese, and therefore were tested.

3.1.1. Growth characteristics

All lactococci (*Lc.* subsp. *lactis* and *cremoris*) were able to grow at 10, 30 and 40 °C and in the presence of 1, 2 and 4% NaCl (Tab. I). Forty-six percent of *Lc.* subsp. *lactis* and one strain of *Lc.* subsp. *cremoris* were able to grow in a medium supplemented with 6.5% NaCl, which reflects salt tolerance in comparison with the usual milk-fermenting strains of lactococci [38].

Most lactobacilli (*Lb. rhamnosus*, *Lb. fermentum* and *Lb. plantarum*) were heterofermentative. Eighty percent of *Lb. plantarum* did not produce CO_2, as they are grouped with facultatively heterofermentative lactobacilli [28]. The strains also included a few homofermentative lactobacilli: one *Lb. acidophilus*, 2 *Lb. salivarius*

and 5 *Lb. delbrueckii* subsp. *lactis*. Some mesophilic lactobacilli, e.g. *Lb. paracasei* and *Lb. plantarum* were able to grow at 40 and 45 °C, and the thermophilic lactobacilli strains; one *Lb. acidophilus* and one strain of *Lb. fermentum* were able to grow at 10 °C (Tab. I), which is rather unusual for the standard characteristics of these strains [42]. Ninety-five percent of lactobacilli were salt-tolerant: they were able to grow in a medium supplemented with 6.5% NaCl. The physiological properties of enterococci were found to be typical for this group: for example, salt resistance and growth at 10–45 °C [36].

Salt levels in Ras cheese generally range from 4–6% [3] and during manufacturing the coagulum is cut into small pieces and stirred at 45 °C for 40 min [26]. The ability of lactococci to grow at 40 and 45 °C, and in the presence of 4–6.5% NaCl, should make them functional for application in Ras cheese as primary starter cultures, and lactobacilli as adjunct cultures.

3.1.2. Acidification activity

Forty-three percent of enterococci, 21% of lactobacilli and 20% of lactococci exhibited a medium acidification rate, while one strain of *Lb. delbrueckii* subsp. *lactis* and *E. faecium* exhibited a fast acidifying ability. The majority of strains showed a slow rate of acidification (Tab. II and Fig. 1). These results are in agreement with Ayad [4], who indicated that the acidifying activity of several wild lactococci is rather low; and with Sarantinopoulos et al. [36] who found that *E. faecium, E. faecalis* and *E. durans* strains were poor acidifiers in milk. Most tested strains of *Lb. plantarum, Lb. rhamnosus*, *Lb. fermentum* and *Lb. pentosus* showed a slow acidification rate, as they are grouped with facultatively heterofermentative lactobacilli [28]. Since a rapid decrease in pH during the initial step of cheese preparation is important for coagulation and the reduction of the growth of adventitious microflora, the fast- and medium-acidifying strains are useful as primary starters, whereas the poor acidifier strains can be used as adjunct cultures depending on their other properties.

3.1.3. Aminopeptidase (AP) activity

Aminopeptidase activity was determined to express the proteolytic activity of strains, and generally was found to be higher for most lactobacilli (1–100 unit OD_{650}^{-1}) compared with lactococci and pediococci (1–40 unit OD_{650}^{-1}) and enterococci (0.8–19 unit OD_{650}^{-1}). The AP was divided into good, fair and poor according to the activity level of each genus (Tab. II and Fig. 1). The highest AP activity was detected in *Lb. rhamnosus*, *Lb. plantarum, Lb. casei* and *Lb. pentosus*. These results are in agreement with Dako et al. [11], who reported that peptidase activities of lactobacilli were generally higher compared with lactococci. *L. lactis* subsp. *lactis* showed AP activity higher than *L. lactis* subsp. *cremoris.* Forty-seven percent of lactococci, 29% of lactobacilli and 18% of enterococci strains exhibited good and fair AP activity (Fig. 1); those strains may have an impact during the ripening of Ras cheese. However, most peptidases have been found to be intracellular [30], reflecting the importance of cell lysis.

3.1.4. Autolytic activity

There was a wide diversity in the autolytic activity of tested strains, which led to the classification of the cultures into three groups; poor, fair, and good, according to the autolytic capacity of each genus (Tab. II and Fig. 1). Lactobacilli showed a higher autolysis rate (0–96%) when compared with enterococci (0–66%) or lactococci strains (1–37%). These results are comparable with the findings of El-Soda et al. [19], who reported that the autolytic activity of *Lactobacillus* was higher than other LAB. Forty percent of lactococci, mainly of subsp. *lactis*, had a fair autolytic activity and the rest of the strains showed poor autolysis. These results are in agreement with Ayad [4], who indicated that several wild lactococci were stable in milk cultures and during cheese ripening. Although the wild lactococci are stable, they harbour active amino-acid-converting enzymes which play a key role in the formation of amino-acid-derived flavour components [5]. Smit et al. [37] reported that the activity

Table II. Technological characteristics of strains.

Strains	Total numbers	Acid production[a]			Aminopeptidase activity (AP) [b]			Autolytic activity[c]			Strains producing bacteriocins	Strains producing EPS
		Fast	Medium	Slow	Good	Fair	Poor	Good	Fair	Poor		
Lactococcus												
L. lactis subsp. *lactis*	13	–	3	10	9	–	6	–	6	7	9	–
L. lactis subsp. *cremoris*	2	–	–	2	1	–	1	–	1	1	1	–
Lactobacillus												
Lb. delbrueckii subsp. *lactis*	5	1	2	2	–	–	3	1	3	1	–	–
Lb. fermentum	16	–	1	15	2	3	16	7	4	5	2	3
Lb. rhamnosus	31	–	8	23	6	–	14	7	12	12	6	–
Lb. paracasei subsp. *paracasei*	6	–	3	3	3	1	4	2	3	1	3	1
Lb. pentosus	10	–	2	8	3	3	6	–	7	3	3	3
Lb. plantarum	21	–	3	18	1	–	17	4	13	4	1	–
Lb. brevis	3	–	–	3	–	–	3	–	3	–	–	–
Lb. acidophilus	1	–	1	–	–	–	–	–	–	1	–	–
Lb. salivarius	2	–	1	1	–	1	2	–	1	1	–	1
Enterococcus												
E. faecium	59	1	28	30	20	–	49	2	10	47	20	–
E. durans	5	–	–	5	2	–	5	–	2	3	2	–
E. faecalis	9	–	5	4	1	–	6	2	4	3	1	–
E. avium	2	–	–	2	2	–	1	–	1	1	2	–
E. casseliflavus	2	–	–	2	–	–	2	–	1	1	–	–
Pediococcus												
Ped. pentosaceus	1	–	–	1	–	–	1	1	–	–	–	–

[a] Fast, medium and slow; when a pH of 0.4 units was achieved after 3, 3–5 and >5 h, respectively.
[b] AP activity level of lactococci and pediococci: good, 20–40; fair, 10–19; poor, 0–9. Lactobacilli: good, 60–100; fair, 30–59; poor, 1–29 and enterococci: good, 13–19; fair, 6–13; poor, 0.8–5.
[c] Autolytic activity level of lactococci and pediococci: good, 25–37; fair, 15–24; poor, 1–14. Lactobacilli: good, 70–96; fair, 40–69; poor, 0–39 and enterococci: good, 35–66; fair, 24–34; poor, 0–23.

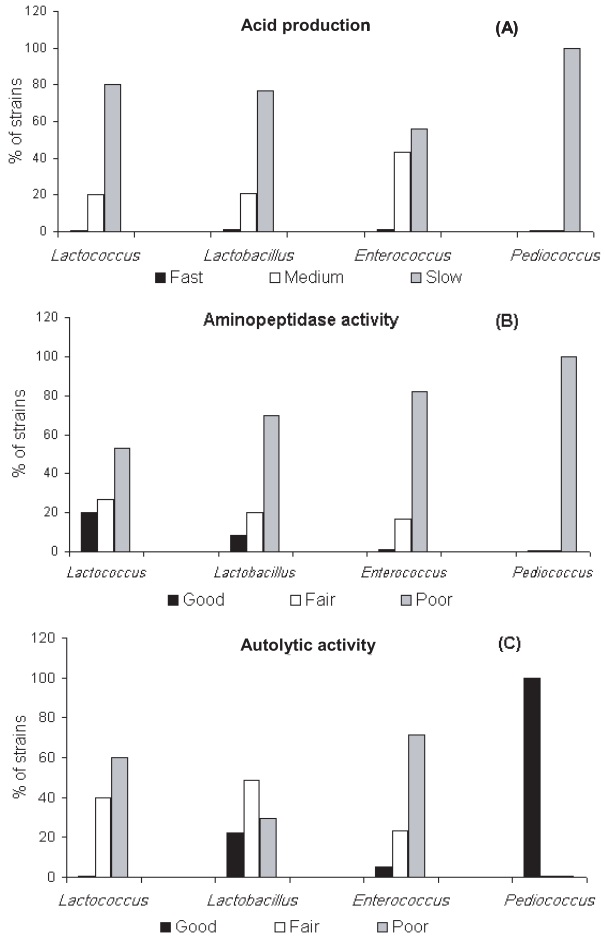

Figure 1. Percentage of strains according to their technological characteristics. (A) fast-, medium- and slow-acidifying; when a ΔpH of 0.4 units was achieved after 3, 3–5 and >5 h, respectively. (B) AP activity level of lactococci and pediococci: good, 20–40; fair, 10–19; poor, 0–9. Lactobacilli: good, 60–100; fair, 30–59; poor, 1–29 and enterococci: good, 13–19; fair, 6–13; poor, 0.8–5. (C) autolytic activity level of lactococci and pediococci: good, 25–37; fair, 15–24; poor, 1–14. Lactobacilli: good, 70–96; fair, 40–69; poor, 0–39 and enterococci: good, 35–66; fair, 24–34; poor, 0–22.

of peptidases (enzymes) that require cofactors might be negatively affected by lysis of the cell. It probably depends on the type of enzyme (system) whether lysis will improve the activity and formation of flavour or not. In cheese practice, a balance in starter autolysis should be necessary for optimal cheese flavour development.

3.1.5. Antagonistic activity

Twenty-seven percent of the tested strains exhibited antimicrobial activity; 6% *Lactococcus*, 8% *Lactobacillus* and 13% of *Enterococcus* strains (Tab. II). Many LAB are able to produce bacteriocins against the same species or closely related species [27];

this antimicrobial activity is probably a consequence of bacteriocin production. Sixty-seven percent of *Lactococcus* were bacteriocin-producing; 60% of these strains were subsp. *lactis* and 7% were subsp. *cremoris*. *Lactobacillus*-producing strains were *Lb. rhamnosus* (6.3%), *Lb. pentosus* (3.1%), *Lb. paracasei* (3.1%), *Lb. fermentum* (2.1%) and one strain of *Lb. plantarum*. Thirty-two percent of enterococci exhibited antagonistic activity: *E. faecium* (26%), *E. durans* (3%), *E. avium* (3%) and one strain of *E. faecalis.* Bacteriocin-producing strains have been used in starter cultures to improve the safety and quality of the cheese [12] and recently used with sensitive adjunct cultures to increase their autolysis in order to accelerate cheese ripening [35]. Further work should identify the antimicrobial compounds produced by strains before applying them in Ras cheese.

3.1.6. Exopolysaccharides (EPS) production

Production of EPS is a feature of many strains of LAB which may be bound to the bacterial cell as capsules or diffuse away from the cell as an extracellular slime [10]: 4.3% of the tested strains were capsule producers, all belonging to lactobacilli (Tab. II). The EPS-forming LAB are used in the dairy industry as a natural biothickener to enhance the rheological quality [25]. The EPS-producing strains will be used to improve the manufacture of low fat cheese to obtain a better mouth feeling, and in Egyptian fermented milks.

3.2. Production characteristics of strains

In order to consider any strain as a starter culture, it should meet a number of criteria for the production of the biomass; high yield in fermentation has to be retained easily by centrifugation or microfiltration and must resist lyophilisation with little practical loss of activity if it will distribute as freeze-drying cultures [9]. Most tested strains were able to produce high yield (good and fair) of biomass in fermentation (Tab. III and Fig. 2). *Lactobacillus* strains (85%) had generally the highest CDW ranging from 1.0 to 2.30 $mg \cdot L^{-1}$; *Lb. rhamnosus*, *Lb. plantarum*, *Lb. fermentum* and *E. faecium* exhibited high biomass production compared with the others.

The strains showed a good biomass separation when collected by centrifugation; 93% of *Lactococcus*, 89% of *Lactobacillus* and 97% of *Enterococcus* showed a well-formed pellet (e.g., good and fair): see Table III and Figure 2.

The activity of 67% of *Lactococcus*, 63% of *Lactobacillus* and 77% of *Enterococcus* strains was not affected by lyophilisation (Tab. III and Fig. 2). The enterococci had the greatest ability to endure lyophilisation, and *E. faecium* strains were the most resistant strains (60%).

3.3. Flavour of selected strains

The strains which showed outstanding characteristics were individually grown in milk cultures to determine their flavour-forming abilities. Most strains appeared to produce pleasant flavours; e.g., yoghurt-like, sour and creamy, which are usual flavours for fermented milks. Some strains produced different flavours compared with those usual flavours described as specific flavours, such as fruity, fatty acids, sharp, yeasty, sweet, esters, malty, sulphur, old cheese-like, Ras cheese-like, etc. (Tab. IV). Each of the selected strains was found to be able to produce the same flavour attributes in Ras cheese slurries (under publication). The flavour profile of ripened cheese is mainly affected by proteolysis of caseins and in some types also by lipolysis [2] and the typical cheese flavour results from further conversion of amino acids by enzymes of starter bacteria [8]. In our previous study [6], several market artisanal Ras cheese samples, the origin of whose LAB were tested in the present study, were sensorially evaluated and the flavour volatile compounds were identified using purge-and-trap thermal desorption cold-trap gas chromatography mass spectrometry. Sixty-eight volatile compounds have been identified as being responsible for the typical flavour of cheese. The flavour of artisanal cheese is influenced by the associated wild flora

Table III. Production characteristics and selected strains.

Strains	Total numbers	Cell dry weight (mg·L^{-1}) [a]			Separation of biomass [b]			Stability of freeze-drying cultures[c]			Number of selected strains[d]
		Good	Fair	Poor	Good	Fair	Poor	Good	Fair	Poor	
Lactococcus					5						
L. lactis subsp. *lactis*	13	2	6	3	–	3	1	3	5	5	6
L. lactis subsp. *cremoris*	2	1	1	–		–	–	–	2	–	1
Lactobacillus					2						
Lb. delbrueckii subsp. *lactis*	5	2	2	2	9	2	–	2	1	2	3
Lb. fermentum	16	12	3	4	11	4	1	4	3	9	10
Lb. rhamnosus	31	10	16	7	3	7	6	7	13	11	15
Lb. paracasei subsp. *paracasei*	6	3	2	2	3	2	–	2	3	1	5
Lb. pentosus	10	1	8	3	9	3	1	3	4	3	7
Lb. plantarum	21	8	10	7	–	7	2	7	5	9	11
Lb. brevis	3	2	–	3	–	3	1	3	–	–	1
Lb. acidophilus	1	–	1	1	1	1	–	1	–	–	–
Lb. salivarius	2	1	1	1		1	–	1	–	1	–
Enterococcus					13						
E. faecium	59	13	35	19	1	19	1	19	27	13	23
E. durans	5	1	3	2	4	2	1	2	2	1	2
E. faecalis	9	4	3	3	1	3	–	3	4	2	7
E. avium	2	1	1	–	1	–	–	–	1	1	–
E. casseliflavus	2	1	–	–	–	–	–	–	1	1	–

[a] Cell dry weight (mg·L^{-1}), good, ≥ 1.3 mg·L^{-1}; fair, 1.29–0.6 mg·L^{-1}; poor, < 0.6 mg·L^{-1}.
[b] Good, (0–0.1 OD_{650}); fair, (0.2–0.3 OD_{650}); poor > 0.3 $OD_{650;}$ OD_{650} of the growth media after centrifugation.
[c] Good ≤ 1 h; fair ≤ 1.5 h; poor, > 2 h; time (h) needed to reduce the pH by 0.4 units of the freeze-dried culture.
[d] Selection according to production and technological criteria.

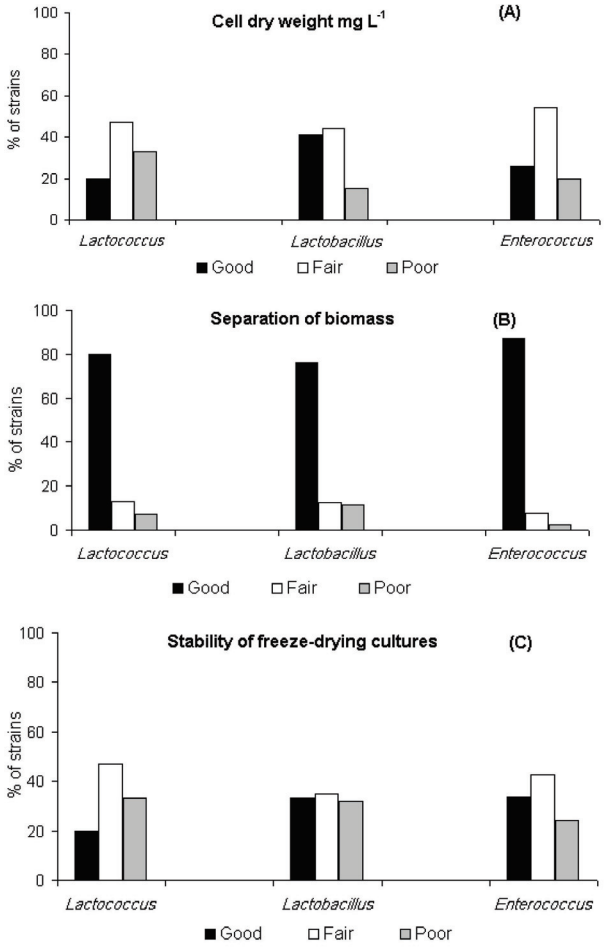

Figure 2. Percentage of strains according to their production characteristics. (A) cell dry weight (CDW mg·L^{-1}), good, ≥ 1.3 mg·L^{-1}; fair, 1.29–0.6 mg L^{-1}; poor, < 0.6 mg·L^{-1}. (B) the OD_{650} of the growth media after centrifugation was used to express the ability of biomass separation; good, (0–0.1 OD_{650}); fair, (0.2–0.3 OD_{650}); poor > 0.3 OD_{650}. (C) the effect of lyophilisation on the culture stability was determined as the difference in time needed to reduce the pH of reconstituted milk by 0.4 units of the freeze-dried and unfreeze-dried culture; good, ≤ 1h; fair, ≤ 1.5 h; poor, > 2 h.

which include mesophilic and thermophilic LAB and the flavour-forming abilities are varied between these microorganisms [32]. The tested LAB harbour active amino acid convertases which could explain their ability to produce different flavours in milk and in cheese slurries. Therefore, the use of these strains to produce typical Ras cheese from pasteurised milk looks promising.

Ninety-one strains; 7 *Lactococcus*, 52 *Lactobacillus* and 32 *Enterococcus* (Tab. III) were selected according to their production and technological criteria relevant for an application in Ras cheese manufacture. As an example, the characteristics of 37 selected strains are presented in Table IV. Some strains were able to maintain a high activity of two or more technological characteristics together, e.g., two

Table IV. Characteristics and flavour profile of some selected strains.

Strains	Species	Acid production[a]	AP activity[a]	Autolytic activity[a]	Growth in 6.5% salt	Bacteriocin production	EPS	Flavour description[b]
Lactococcus								
FAAU 17L	*L.* subsp. *lactis*	Medium	Good	Fair	+	+	–	Sharp, acid (4), yoghurt-like
FAAU 19L	*L.* subsp. *lactis*	Slow	Good	Fair	+	+	–	Cheese-like, flat
FAAU 67L	*L.* subsp. *lactis*	Medium	Fair	Fair	+	+	–	Sweet (1), Laban Rayeb-like[c]
FAAU 71L	*L.* subsp. *lactis*	Slow	Good	Fair	–	+	–	Strong, acid (3), yoghurt-like
FAAU 1M	*L.* subsp. *lactis*	Medium	Poor	Fair	–	+	–	Yeasty, sharp, acid (3), cheese-like
FAAU 2M	*L.* subsp. *lactis*	Slow	Fair	Poor	–	–	–	Flat, fermented milk-like
FAAU 6M	*L.* subsp. *cremoris*	Slow	Good	Fair	+	–	–	Diacetyl, ester, fruity (1)
Lactobacillus								
FAAU 119th	*delb.* subsp. *lactis*	Fast	Fair	Good	+	–	–	Sour (4), fatty (2), yoghurt-like
FAAU 121th	*delb.* subsp. *lactis*	Fast	Fair	Good	+	–	–	Yoghurt-like, acid (3), sweet (1)
FAAU 93th	*fermentum*	Fast	Fair	Good	+	–	–	Sharp, acid (4), Laban Rayeb-like
FAAU 2ka	*fermentum*	Slow	Poor	Fair	+	–	+	Old cheese-like, bitter (2), gassy
FAAU 53R	*fermentum*	Medium	Poor	Good	+	–	–	Acid (1), Ras cheese-like
FAAU 41M	*fermentum*	Slow	Poor	Good	+	+	–	Farm cheese-like
FAAU 5M2	*rhamnosus*	Slow	Good	Fair	+	–	+	Creamy, slimy (2), acid (3), ester
FAAU 91st	*rhamnosus*	Slow	Fair	Fair	+	–	–	Farm cheese-like
FAAU 97st	*rhamnosus*	Medium	Fair	Poor	+	–	–	Gassy, sour(2), cheese-like
FAAU 115st	*rhamnosus*	Medium	Good	Fair	+	+	–	Laban Rayeb-like, acid (1)
FAAU 48R	*para.* subsp. *pracasei*	Medium	Poor	Fair	+	+	–	Mild yoghurt, sour (2)
FAAU 36M	*para.* subsp. *pracasei*	Medium	Good	Fair	+	+	+	Ras cheese-like, sweet (1), acid (1)
FAAU 105st	*pentosus*	Slow	poor	Fair	+	–	–	Sulphur, gassy, acid (1), farm cheese
FAAU 123st	*pentosus*	Slow	poor	Fair	+	–	–	Farm cheese-like, acid (1)
FAAU 117st	*plantarum*	Medium	Fair	Fair	+	+	–	Malty (1), sour (4)
FAAU 118st	*plantarum*	Medium	Poor	Fair	+	–	–	Gassy, acid (1)
FAAU 40M	*plantarum*	Slow	Poor	Good	+	–	–	Creamy, sweet (1), flat
FAAU 98st	*plantarum*	Slow	Poor	Fair	+	–	–	Fresh yoghurt-like, creamy (1)
FAAU 127st	*brevis*	Slow	poor	Fair	+	–	–	Yoghurt-like, acid (2)
FAAU 132st	*acidophilus*	Medium	Good	poor	+	–	–	Old yoghurt-like, strong acid (4)
Enterococcus								
FAAU 291E	*faecium*	Slow	Good	Fair	+	–	–	Sharp, gassy, Ras cheese-like
FAAU 398E	*faecium*	Medium	Fair	Poor	+	+	–	Yoghurt-like
FAAU 405E	*faecium*	Medium	Fair	Poor	+	+	–	Sweet (2), fatty (1), Laban-Rayeb-like
FAAU 411E	*faecium*	Fast	Poor	Fair	+	–	–	Yoghurt-like, sweet (1), fatty (1)
FAAU 361E	*durans*	Slow	Poor	Fair	+	+	–	Sulphur (1), bitter (2)
FAAU 374E	*durans*	Slow	Poor	Fair	+	+	–	Flat, sweet (2), Laban Rayeb-like
FAAU 280E	*avium*	Slow	Fair	Fair	+	–	–	Flat, yoghurt-like
FAAU 371E	*faecalis*	Medium	Fair	Good	+	+	–	Mild yoghurt, acid (1)
FAAU 404E	*faecalis*	Medium	Fair	Good	+	–	–	Sweet (1), yoghurt-like
FAAU 452E	*casseliflavus*	Slow	Poor	Fair	+	–	–	Fresh curd, yoghurt-like

[a] Acid production, AP and autolytic activity (for level definitions see legends of Tab. II and Fig. 1).
[b] Flavour intensity on scale from (1–4): 1: slightly, 2: moderate, 3: strong, 4: very strong.
[c] Kind of Egyptian fermented milk (for description see El-Gendy, [16]).

strains, *Lb. fermentum* (41M) and *E. faecalis* (371E), exhibited good autolytic activity and showed an antagonistic effect. Five strains, 3 *Lc. lactis* subsp. l*actis* (17L, 19L and 71L), *Lb. rhamnosus* (5M2) and *Lb. paracasei* subsp. *paracasei* (36M), were able to produce high AP activity and showed an antagonistic effect. Three strains, 2 *Lb. delbrueckii* subsp. *lactis* (119th and 121th) and *Lb. fermentum* (93th) showed a fast acidification, good autolytic activity and fair AP activity. Moreover, the *Lb. paracasei* subsp. *paracasei* (36M) was able to produce bacteriocin, EPS and had high AP, fair autolytic activity and medium acidification activity. Two lactococci strains, 17L and 67L, were salt-tolerant (6.5% NaCl), showed antagonistic effect, good AP activity and fair autolytic activity. These strains are valuable for practical application for different purposes, as starter, adjunct and protective cultures.

4. CONCLUSION

Our investigation discovered the pool of LAB associated with traditional Ras cheese, and their biochemical and production properties, which are relevant to their application as starter cultures. Ninety-one strains: 7 *Lactococcus*, 52 *Lactobacillus* and 32 *Enterococcus,* were selected and could be used in Ras cheese manufacture according to their technological criteria, salt tolerance, ability to grow at 40–45 °C, acid production, proteolytic activity, flavour and bacteriocin production. The production criteria were also taken into account: all selected strains were resistant to lyophilisation conditions, showed high yield of biomass in fermentation and a good separation after centrifugation. Selected strains are currently being investigated in pilot-scale cheese trials as single and mixed cultures to improve the quality and safety of Ras cheese.

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